E-UNIT B2–2

Asexual Propagation

Imagine coming across a single very unusual apple tree with large blue fruit that has outstanding flavor. You would like to mass-produce the tree. How would you go about creating many other trees that produced the exact fruit? If you were to rely on the seed, very possibly the blue color or outstanding flavor could be lost. The answer is in asexual propagation.

Objective:

Describe asexual propagation methods.

Key Terms:

adventitious root	endogenous root	plantlets
agar	exogenous root	subculture
asexual propagation	explants	tissue culture
callus	meristematic tissue	totipotency
dedifferentiation	meristemoid	

Asexual Reproduction of Plants

Asexual propagation is the reproduction of new plants using only the vegetative parts of the parent plant. Unlike sexual reproduction, asexual reproduction results in offspring that are the genetic duplicates, or clones, of the parent plant.

Some advantages are associated with asexual reproduction. Plants with outstanding characteristics can be produced without the risk of losing the desired characteristics

through the recombination of genes. Some plants are difficult to reproduce sexually because they produce few seeds or because the seeds they produce have low germination rates. A big advantage of asexual reproduction is that huge numbers of genetically identical plants can be produced. Also, mature plants can be obtained more rapidly than those grown from seed.

ADVENTITIOUS ROOT FORMATION

Asexual propagation is most commonly concerned with the regeneration of roots. Plant parts removed from the parent plant are treated to encourage the development of adventitious roots. An **adventitious root** is a root that arises from any plant part other than by the normal development of seedling roots or their branches.

The development of adventitious roots is a complex physiological process. The speed of root development is influenced by various factors, including the plant species or variety, the age of the plant, the type and location of the cutting, the absence or presence of leaves, and the nutritional status of the plant. Woody plants tend to take longer to propagate than herbaceous (nonwoody, soft-stem) plants.

Wounding contributes to the formation of adventitious roots. When a wound is made, jasmonic acid is produced by the plant as a stress response to reduce further damage from pests and disease organisms. The wound is sealed off by **callus**, or a mass of unorganized parenchyma cells, that forms on a wounded surface. Jasmonic acid also acts as a positive regulator of adventitious root formation.

Adventitious root initiation is promoted by high levels of auxins and low levels of cytokinins. On the other hand, low levels of auxins and high levels of cytokinins promote adventitious bud/shoot development. The application of rooting hormones to cuttings containing auxins enhances the formation of callus in addition to inducing the formation of adventitious roots. The two synthetic root-promoting materials most widely used are naphthaleneacetic acid (NAA) and indolebutyric acid (IBA). IBA is regarded as the best material for general use. It can be used with a wide variety of plants, and it is nontoxic over a wide range of concentrations.

High levels of carbohydrates in the plant tissue fuel adventitious root formation. Actively growing plant tissue has a higher concentration of carbohydrates than plant tissue that is not actively growing. This is one reason cuttings should be taken from healthy actively growing plant material.

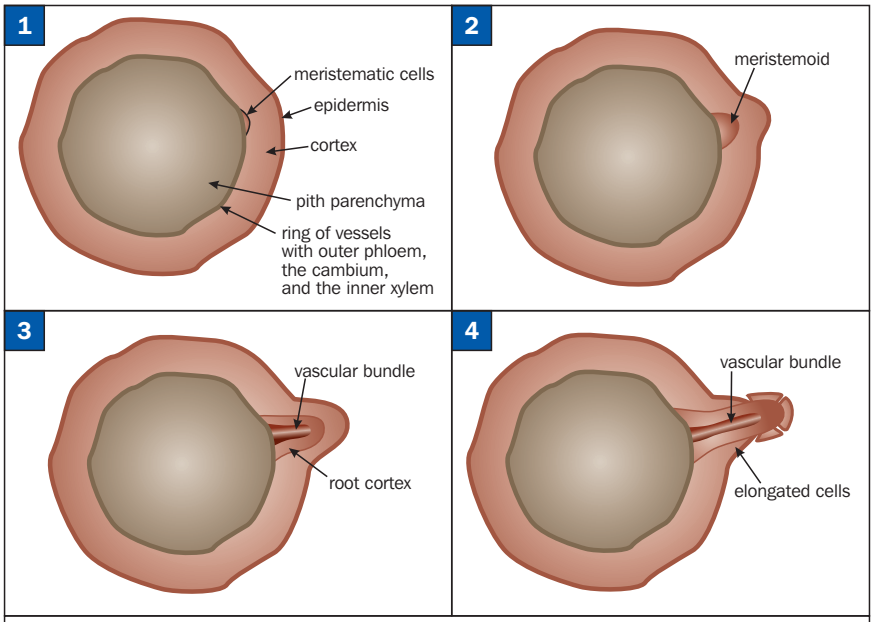
Four Phases of Adventitious Root Formation

Adventitious root formation can be broken into four main phases: dedifferentiation, root initiation, root primordium formation, and root elongation and/or emergence.

- *Phase 1:* Dedifferentiation, or remeristemation, of parenchyma cells takes place. **Dedifferentiation** is the process by which previously developed differentiated cells near the vascular tissues become meristematic tissue. **Meristematic tissue** is plant tissue that contains undifferentiated cells from which new cells form and is located

in plant zones where growth occurs. Cells in the meristematic tissue are totipotent. **Totipotency** is the potential of a cell to differentiate into any type of cell depending on the special function required. Essentially a single totipotent cell can develop into an entire organism.

Some tissue types are not capable of totipotency. Their genomic machinery is permanently dedicated to a given tissue type, and they do not readily dedifferentiate. The types of tissue that lend themselves to totipotency are the meristematic tissue (active growing points of the plant) and embryonic tissue. Roots and leafy material are more difficult to culture.



Phases of adventitious root formation

1. *Dedifferentiation phase:* The first meristematic cells of the developing root appear.
2. *Root initiation phase:* The meristemoid forms.
3. *Root primordium formation phase:* The first well-developed young root meristems become visible, and a backward differentiation of cells of the root body containing root cortex and vascular bundles occurs.
4. *Root elongation and/or emergence phase:* The first root with vascular bundles emerges in the center, surrounded by elongated cells.

FIGURE 1. Adventitious root formation.

- *Phase 2:* The root initiation phase is characterized by root initials consisting of meristematic cells that divide and form slightly organized cell groups. Starch grains are degraded, the first cell divisions take place, and meristemoids appear. A **meristemoid** is a small, triangular stomatal precursor cell that functions temporarily as an undifferentiated stem cell in a meristem. Meristemoids form in the callus area or near vascular bundles.
- *Phase 3:* The development of meristemoids marks the transition from the root initiation phase to the root primordium formation phase. During the root primordium formation phase, the first recognizable young root meristems become visible. Globular structures develop dome-shaped root primordia, which include the meristem followed by the first cells of the root body.
- *Phase 4:* Cells of the root primordia elongate. The first elongated cells of the roots are found in the elongation zone inside the stem but have all the morphology of a complete root except for root hairs. Vascular tissues form and connect the root to the cutting.

Origin of Adventitious Roots

Two major types of root formation are endogenous and exogenous. An **endogenous root** is a type of adventitious root that forms within the stem; root initiation is usually near vascular bundles and near the source of hormones, carbohydrates, etc. The area where the root forms acts as a sink and draws nutrients toward it. An **exogenous root** is a type of adventitious root initiated within callus that forms at the site of a wound or, in the case of a cutting, where the cut was made. Exogenous root formation occurs mostly in hard-to-root plants, including many conifers. Root initials start in callus. The vascular elements form and eventually join with the vascular tissues of the stem.

The location of root initiation differs between herbaceous and woody plants. Adventitious roots originate just outside and between vascular bundles in herbaceous plants. Depending on the type of plant, root initiation may occur from different tissues, such as phloem, parenchyma, epidermal, and pericycle. Adventitious roots in woody perennials originate from living parenchyma cells in young secondary phloem. They may also arise from vascular rays, cambium, phloem, lenticels, and pith.

Pre-formed root initials are present during stem formation. They lie dormant and develop under the proper conditions. Often the root initials are established at the end of the season in the present year's wood and will grow when the shoot is separated from the plant the following season. Sometimes the roots will begin growing while the stem is intact. For example, contact of stems with the ground may trigger the formation of adventitious roots in willows and brambles.

Certain environmental conditions are critical for successful root formation. They include proper temperature, high humidity, and sufficient light. In addition, the rooting medium must be free of disease organisms that could infect the plant and should have both good water-holding ability and good aeration.

ASEXUAL PROPAGATION TECHNIQUES

Asexual propagation techniques include cuttings, grafting, layering, separation, division, and tissue culture.

Cuttings

One of the commonest and simplest methods of asexual propagation is that of cuttings. Cuttings are typically portions of stems or leaves from which new plants are produced.

Stem Cuttings

Stem cuttings include the stem and leaf portions of a plant. Stem cuttings 2 to 5 inches in length are taken in the morning when the tissues are turgid, or full of water. A clean sharp knife or razor should be used for the cuts. Disinfecting the cutting blade between cuts is advisable to avoid spreading disease. Any flowers on the stem of a cutting should be removed to direct energy to the production of roots. In addition, the proximal end of

the cutting, or the end closest to the root system, is usually dipped in a rooting hormone to hasten the formation of adventitious roots. Then the cutting is placed in a rooting cube or directly into a rooting medium.

After cuttings are stuck, they are placed under an intermittent mist system. The mist of water is applied at regular intervals between dawn and sunset to reduce water loss from the plant tissue through transpiration. Misting frequency is reduced once the roots begin to form and can absorb moisture for the plants. The propagation area is often shaded to reduce stress from the sun. Bottom heat may also be provided to maintain a medium temperature between 75°F and 80°F.

The three basic types of stem cuttings are softwood cuttings, semi-hardwood cuttings, and hardwood cuttings. Softwood cuttings are plant pieces taken from soft, succulent growth. Semi-hardwood cuttings are plant pieces taken from woody, broad-leaved plants with new shoots. Hardwood cuttings are plant pieces taken from one-year-old growth of deciduous or evergreen plants.

FIGURE 2. Stem cuttings include the stem and leaf portions of a plant.

Leaf Cuttings

A relatively small number of plants have the ability to produce plantlets on their leaves. In such cases, entire leaves or portions of leaves are removed from the parent plant for use as leaf cuttings. Healthy leaves that have just reached maturity should be used, because the leaves are at a stage when food production and the capacity to produce new plantlets are highest.

Leaf-Petiole Cuttings

Some plants, including African violets, are easily propagated by leaf-petiole cuttings. This type of cutting includes the leaf blade of the parent plant and the petiole. Under proper conditions, a cluster of plantlets develops where the petiole was cut. When the plantlets have grown large enough to handle, they are separated and potted in individual containers.

Leaf-Bud Cuttings

Many plants that cannot be propagated by leaf or leaf-petiole cuttings can be propagated by leaf-bud cuttings. A leaf-bud cutting consists of the leaf blade, the petiole, a bud

at the base of the petiole, and a portion of the stem. The leaf provides the energy for root development. The roots sprout from the stem and are often concentrated at the node of the stem. The bud develops into the stem of the new plant.

Foliar Embryos

A few plants, including the piggyback plant (*Tolmiea menziesii*), kalanchoe, and the strawberry begonia, produce foliar embryos. Through a complex process, cells in small areas of a leaf develop into plantlets that can be removed and planted.

Grafting

Woody plant cultivars are often propagated by grafting. Grafting is the process in which the stem of one plant is made to grow on the roots of another plant.

The scion is the portion of the graft that is to become the stem. The lower portion of the plant that includes the root system is the rootstock or the understock. For the graft to be successful, the cambium wood of the scion and the rootstock must line up. To fuse, the scion and the rootstock materials must also be genetically related. In time, the conductive tissues grow as one. Budding is a form of grafting in which the scion consists of a single bud.

Layering

Layering is a method of asexual reproduction in which roots form on a stem while the stem is still attached to the parent plant. The advantage of layering is that the parent plant provides the plant-to-be with water and minerals until it produces its own roots. Layering is sometimes used with brambles, woody shrubs, and select greenhouse plants.

Separation and Division

Propagation of some plants can be done by separation and division. These are common methods used with herbaceous perennial plants and houseplants. Vegetative plant structures can be removed

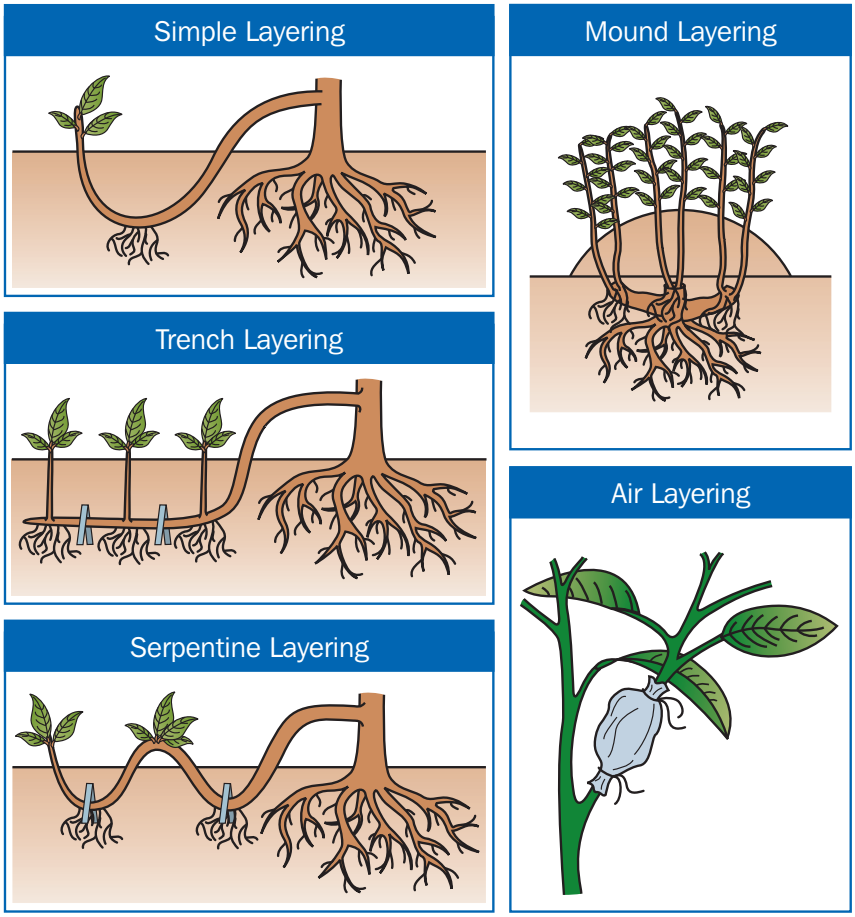


FIGURE 3. Layering is a method of asexual reproduction in which roots form on a stem while the stem is still attached to the parent plant.

intact from some of these plant types. Separation is the process in which these vegetative structures are removed and planted. In contrast, division is the process in which plant roots or an entire plant is cut into sections to make two or more plants from the original plant.

Bulbs are produced off the main bulb, separated, and planted. Some species of lilies produce bulbils, or tiny aboveground bulbs, in the axils of their leaves. The bulbils can be removed and planted. Lilies may produce tiny bulbs below the ground called bulblets. Some lilies and fritillaries can be propagated by removing bulb scales and placing them in a moist medium. In time, they root and produce bulblets that can be separated and planted. Corms, including crocus and gladiola, develop small corms known as cormels. These miniature corms can be separated and planted.

Tissue Culture

Tissue culture is the practice of growing cells or small pieces of plant tissue on artificial media under sterile conditions. Another name for tissue culture is micropropagation. Tissue culture is possible because of totipotency, or the capability of a single cell to develop into an entirely new plant under proper conditions. Small plants or plant shoots that form during tissue culture and are capable of developing into complete plants are called **plantlets**.

Tissue culture is common in research and commercial production. Tissue culture techniques are essential in the development of genetically engineered plants. Special equipment and facilities, as well as highly trained technicians, are required.

FIGURE 4. Orchids are being propagated by tissue culture.

Various aspects make tissue culture advantageous over other propagation methods. For example, tissue culture techniques allow large numbers of plants to be produced from a single plant in a relatively small space and in a short period. This minimizes the need for growing space, reduces labor, and decreases plant maintenance requirements. Viruses and other systemic diseases can be eliminated by propagating the quickly dividing cells of the shoot tip. Tissue culture enables growers to produce plants with identical genetic material.

Occasionally, when plant cells multiply, a change takes place in which the plants vary slightly from the parent plant. Improved agricultural cultivars may result through these chance occurrences. Some characteristics subject to improvement are leaf shape, disease resistance, growth habit, and flower color.

On the Job... *CAREER CONNECTION*

Plant Tissue Culture Technician

Tissue culture is a highly technical method of plant propagation that is widely used today. Consequently, there is a need for plant tissue culture technicians.

Plant tissue culture work is performed in laboratory-type settings, where conditions are sterile. Plant tissue culture technicians must pay careful attention to cleanliness in the work environment. Technicians propagate foliage plants, perennial plants, cut flowers, woody plants, and genetically engineered plants.

Plant tissue culture technicians benefit from taking classes in a horticulture or agriculture program at the high school level. An associate's degree in horticulture, agriculture, or botany improves opportunities. Also, work experience in the agriculture or horticulture field is helpful.

CULTURE MEDIA PREPARATION

Culture media are used for plant tissue culture procedures. The quality of a plant tissue culture medium determines the success of plant cell growth. Essentially, the plant tissue culture medium should contain the same nutrients as required by a whole plant. Plants growing in vitro (in glass) cannot synthesize their own food, so nutrients must be provided.

Medium is formulated for the particular species of plant being cultured and the type of material used for culture (i.e., cells, tissues, organs, protoplasts). It may be solid medium or liquid medium.

Commonly used media contain inorganic nutrients (micronutrients and macronutrients), organic supplements (vitamins, amino acids, organic acids, organic extracts, activated charcoal, and antibiotics), carbon and energy sources (sucrose), growth regulators (auxins, cytokinins, gibberellins, and abscisic acid), solidifying agents (mainly agar), and desired pH (optimal pH for most tissue cultures is in the range of 5.0 to 6.0). A number of media formulas have been developed by researchers and are available for purchase.

Agar is a solidifying agent consisting of a polysaccharide obtained from seaweeds. It is favored because it does not react with media components, it is not digested by plant

enzymes, and it is stable at culture temperature. Also, agar at a concentration of 0.5 to 1.0 percent forms a gel that provides support for growing tissues.

It is essential that the medium be prepared with care and precision. If the medium is not properly formulated, the culture will likely fail.

FIGURE 5. Agar forms a gel that provides support for growing tissues.

Typically, media preparation involves preparation of stock solutions in the range of 10x to 100x concentrations using high-purity chemicals and demineralized water. These stock solutions can be stored frozen and used as required. Most growth regulators are not soluble in water and must first be dissolved in NaOH or alcohol.

The procedure for preparing media is time consuming. Therefore, most plant tissue culture media are commercially prepared and made available as dry powders. The dry powders are dissolved in distilled or demineralized water. Sugar, organic supplements, and agar are added, the pH is adjusted, and the media are diluted to a final volume (usually 1 liter). The basic steps in preparation of a tissue culture medium are:

1. Measure out approximately 80 percent of the final required volume of tissue culture grade water (e.g., 800 ml for a final volume of 1,000 ml). Use a container twice the size of the final volume.
2. While stirring the water, add the powdered medium, and stir until completely dissolved. Heating may be required to bring the powder into the solution.
3. Rinse the original container with a small amount of demineralized water to remove traces of the powder, and add the water to the solution made in step 2.
4. Add desired heat-stable supplements (e.g., sucrose, gelling agent, vitamins, etc.).
5. Add what remains of the 200 ml of demineralized water to bring the medium to the final volume.
6. While stirring, adjust the pH of the medium to the desired level by adding NaOH, HCl, or KOH.
7. If a gelling agent is used, heat until the solution is clear.
8. Dispense the medium into the culture vessels before (or after) autoclaving, according to your application.

9. Sterilize the medium in an autoclave at 121°C and 15 psi for 20 minutes or for the time described under protocols for the specific medium.
10. Filter-sterilize and add hormones and other heat-sensitive organic compounds to the medium after autoclaving.
11. Allow the medium to cool before using.

TISSUE CULTURE PROCESSES

The tissue culture propagation process can be defined in four main stages.

In the first stage, small pieces of plant material, called **explants**, are carefully removed from the parent plant. Explants are obtained from the actively growing parts (e.g., shoot tips; sections of leaves, stems, or roots; or embryos) of a desired plant. The explants are cleaned and placed on sterile agar medium in glass containers. Glass bottles, jars, and test tubes are commonly used. Water and nutrients in the medium enter the explants directly through the cell walls.

In the second stage, the cells of the explants multiply in one of two ways. The cells may form callus, which in tissue culture is a group of cells with no particular function. Given the right hormones in the medium, callus cells differentiate and develop into small plantlets consisting of leaves and stems. The other possibility in stage 2 involves the rapid multiplication of plantlets.

Multiplication of explants is accomplished by placing cytokinins in the medium. Cytokinins are hormones responsible for cell division and differentiation. They encourage adventitious growth, which is seen as an increase in the number of buds (usually six to eight per shoot) on the explants. Each bud is capable of becoming a plant and producing more buds. Branching occurs as these buds develop into plant shoots, or plantlets. Technicians divide these new plantlets and transfer them to new containers. (A group of cultured cells or tissue transferred to fresh medium during the tissue culture process is called a **subculture**.) In this way, a single explant can produce millions of plantlets in a year.

The third stage involves the formation of roots. When the plantlets have developed and there is a desire for them to generate roots, they are separated and transplanted to another medium that contains higher levels of auxins to promote root formation. The plantlets are also given higher light intensity in preparation for stage 4.

In the fourth stage, the plantlets are prepared for normal growing conditions. They are removed from glass

FIGURE 6. Plantlets are separated and transplanted to another medium.

containers. The growing medium is gently washed from the plant roots to reduce the growth of potentially harmful bacteria or fungi. The plantlets are divided, planted in a sterile growing medium, and placed in a greenhouse. Since the humidity in the greenhouse is much lower than in glass containers, care must be taken during this transition to acclimatize the plants to their new environment. A common practice is to place the young plants under an intermittent misting system until they grow accustomed to the environment and develop stronger root systems.

Sterile Technique

One of the most important aspects of tissue culture is sterile technique. Sterile technique is the maintenance of an environment that is free of bacteria, fungi, and viruses. Sterilization of agar medium is essential. In addition, the slightest air movement can stir spores of bacteria and fungi and contaminate the agar. Therefore, work is performed at a sterile workstation under a laminar airflow hood that filters bacterial and fungal spores from the air. A laminar airflow hood draws air through a filter and gently blows the air toward the user.

Technicians scrub much like surgeons do before surgery. Media, tools, and bottles or jars are autoclaved. Autoclaving involves the heating of the materials to 245°F for 15 minutes to kill all bacteria and fungi.

Explants must be cleaned before being placed in the culture. Cleaning of the plants before removal of the explants is usually accomplished by a brief soaking in a bleach solution, followed by a rinse with sterile water. Microorganisms introduced with the plant material can grow at rapid rates on the agar. If contamination of the medium occurs, the contents of the containers are discarded.

The transfer of cultures from one container to another at various stages in their development must occur under sterile conditions to prevent contamination by microorganisms.

Summary:

The development of adventitious roots is a complex physiological process. Adventitious root formation can be broken into four main phases: dedifferentiation, root initiation, root primordium formation, and root elongation and/or emergence. Certain environmental conditions are critical for successful root formation. They include proper temperature, high humidity, and sufficient light.

One of the commonest and simplest methods of asexual propagation is that of cuttings. Cuttings are typically portions of stems or leaves from which new plants are produced. Grafting, layering, separation, division, and tissue culture are other common methods of asexual propagation.

Tissue culture is the practice of growing cells or small pieces of plant tissue on artificial media under sterile conditions. The tissue culture propagation process can be defined in four main stages. In the first stage, small pieces of plant material, called explants, are

carefully removed from the parent plant. In the second stage, the cells of the explants multiply to form callus, or callus cells differentiate and develop into small plantlets. The third stage involves the formation of roots. In the fourth stage, the plantlets are prepared for normal growing conditions.

Culture media is used for plant tissue culture procedures. Commonly used media contain inorganic nutrients, organic supplements, carbon and energy sources, growth regulators, solidifying agents, and desired pH.

One of the most important aspects of tissue culture is sterile technique, or the maintenance of an environment that is free of bacteria, fungi, and viruses.

Expanding Your Knowledge:

Practice different asexual propagation techniques at school and at home. Evaluate the results, and analyze the effectiveness of the different methods and how certain plant materials are easier to propagate than others.

Checking Your Knowledge:

Matching

Instructions: Match each term with its correct definition.

a. adventitious root
b. agar
c. callus
d. dedifferentiation
e. endogenous root
f. exogenous root
g. explants
h. meristematic tissue
i. meristemoid
j. totipotency

_____1. small pieces of plant material carefully removed from the parent plant

_____2. a solidifying agent consisting of a polysaccharide obtained from seaweeds

_____3. a mass of unorganized parenchyma cells that forms on a wounded surface

_____4. a small, triangular stomatal precursor cell that functions temporarily as an undifferentiated stem cell in a meristem

_____5. a type of adventitious root initiated within callus that forms at the site of a wound or, in the case of a cutting, where the cut was made

_____6. plant tissue that contains undifferentiated cells from which new cells form and is located in plant zones where growth occurs

_____7. a type of adventitious root that forms within the stem; root initiation is usually near vascular bundles and near the source of hormones, carbohydrates, etc.

_____8. the potential of a cell to differentiate into any type of cell depending on the special function required

_____9. a root that arises from any plant part other than by the normal development of seedling roots or their branches

_____10. the process by which previously developed differentiated cells near the vascular tissues become meristematic tissue

Multiple Choice

Instructions: Select the best answer to each question.

1. What is a method of asexual reproduction in which roots form on a stem while the stem is still attached to the parent plant?
   a. budding
   b. grafting
   c. layering
   d. tissue culture

2. What does a leaf-bud cutting involve?
   a. a leaf blade
   b. a leaf blade with petiole
   c. a leaf blade with petiole and bud and a short piece of stem
   d. woody broad-leaved plants with new shoots

3. _____ cuttings are made from one-year-old growth of deciduous or evergreen plants.
   a. Hardwood
   b. Mature wood
   c. Semi-hardwood
   d. Softwood

4. What is a very technical method of asexual propagation that involves the growing of plant cells or tissues on artificial media under sterile conditions?
   a. division
   b. grafting
   c. separation
   d. tissue culture

5. What condition must be met for a graft to be successful?
   a. The cambium wood of the scion and the rootstock must line up.
   b. The graft must be allowed to dry to kill infectious microorganisms.
   c. The scion and the stock must be from unrelated plants.
   d. The grafting procedure must be done when the scion is dormant and the rootstock is actively growing.
6. What is the process in which plant roots or an entire plant is cut into sections to make two or more plants from the original plant?
   a. division
   b. foliar embryos
   c. grafting
   d. tissue culture

Short Answer

Instructions: Briefly describe each of the following.

1. leaf cutting

2. stem cutting

3. separation

4. budding

5. tissue culture